

PATENT APPLICATION

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for

BIOSENSOR

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BIOSENSOR

FIELD OF THE INVENTION

The present invention relates to a biosensor, more particularly to an  
5 electrochemical biosensor with a continuous capillary channel extending across  
electrodes.

BACKGROUND AND SUMMARY OF THE INVENTION

Electrochemical biosensors are known. They have been used to determine the  
10 concentration of various analytes from biological samples, particularly from blood.  
Electrochemical biosensors are described in U.S. Patent Nos. 5,413,690; 5,762,770;  
5,798,031; and 5,997,817 the disclosure of each of which is expressly incorporated  
herein by reference.

According to the present invention a biosensor is provided. The biosensor  
15 comprises a support substrate having first and second ends, electrodes positioned on  
the support substrate, the electrodes cooperating with one another to define electrode  
arrays situated adjacent to the first end, a spacer substrate positioned on the support  
substrate, and a cover positioned on the spacer substrate. The cover cooperates with  
the support substrate to define a channel a cover positioned on the spacer substrate,  
20 the cover cooperating with the support substrate to define a channel including a  
sample inlet adjacent to the first end and opposite ends. Each electrode array is  
positioned in the channel adjacent to one of the ends.

According to another aspect of the invention, a biosensor is provided. The  
biosensor comprises a support substrate, a first electrode set positioned on the support  
25 substrate, a second electrode set positioned on the support substrate, the first and  
second electrode sets being spaced-apart from one another, and a cover extending  
across the first and second electrode sets. The cover cooperates with the support  
substrate to define a generally linear capillary channel having opposing first and  
second ends and an inlet positioned between the ends and between the first and  
30 second electrode sets.

According to still another aspect of the invention, a method of forming a  
biosensor is provided. The method comprises the steps of forming spaced-apart  
electrode arrays on a surface of a support substrate, placing a spacer substrate on the

support substrate across the electrode arrays, removing a portion of the spacer substrate to expose the electrode arrays, placing a cover on the spacer substrate to define a capillary channel having opposite ends and extending across the electrode arrays, and punching a notch through the support substrate, a portion of the spacer substrate, and the cover to form an inlet to the capillary channel so that the inlet is positioned between the opposite ends.

Additional features of the invention will become apparent to those skilled in the art upon consideration of the following detailed description of the preferred embodiment exemplifying the best mode of carrying out the invention as presently perceived.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The detailed description particularly refers to the accompanying figures in which:

15 Fig. 1A is a perspective view of a biosensor in accordance with the present invention, showing the biosensor including a support substrate and a cover, each of which is formed to include a notch therein.

Fig. 1B is an enlarged perspective view of the biosensor of Fig. 1A with portions broken away.

20 Fig. 2 is an exploded assembly view of the biosensor of Fig. 1, showing the biosensor including two spaced-apart electrode arrays positioned at one end, a spacer substrate including first, second, and third members and formed to extend about the electrode arrays.

Fig. 3 is a view taken along lines 3-3 of Fig. 1A.

25 Fig. 4 is a view taken along lines 4-4 of Fig. 1A.

Fig. 5 is a diagrammatic view showing a manufacturing process for assembling the biosensor of Fig. 1.

#### DETAILED DESCRIPTION OF THE INVENTION

30 The present invention relates to a biosensor and a method for manufacturing a biosensor that provides a manufacturer with precise and accurate dual channel position. The biosensor uses a continuous process to form a channel that extends across electrodes. Aspects of the invention are presented in Figs. 1-5, which are not drawn to scale and wherein like components in the several views are numbered alike.

Figs. 1-4 illustrate an aspect of the invention in the form of a biosensor 10 having an electrode-support substrate 12, an electrical conductor 13 positioned on the substrate 12 that is disrupted to define electrodes 14, 16, 18, a spacer substrate 20 positioned on substrate 12, and a cover 22 positioned on the spacer substrate 20.

5 Biosensor 10 is preferably rectangular in shape. It is appreciated, however, that biosensor 10 can assume any number of shapes in accordance with this disclosure. Biosensor 10 is preferably produced from rolls of material, however, it is understood that biosensor 10 can be constructed from individual sheets in accordance with this disclosure. Thus, the selection of materials for the construction of biosensor 10

10 necessitates the use of materials that are sufficiently flexible for roll processing, but which are still rigid enough to give a useful stiffness to finished biosensor 10.

The electrode-support substrate 12 is shown in Figs. 2 and 3. Referring to Fig. 3, substrate 12 includes a first surface 24 facing the spacer substrate 20 and a second surface 26. In addition, substrate 12 has opposite first and second ends 28, 30 and 15 opposite edges 32, 34 extending between the first and second ends 28, 30. See Fig. 2. The first end 28 includes a notch 36 formed therein. A generally concave-shaped border 38 defines the notch 36. It is appreciated that the notch can take on a variety of shapes and sizes in accordance with this disclosure. Substrate 12 is generally rectangular in shape, it is appreciated, however, that support may be formed in a 20 variety of shapes and sizes in accordance with this disclosure. Substrate 12 is formed of a flexible polymer and preferably from a flexible polymer and preferably from a polymer such as a polyester or polyimide, polyethylene naphthalate (PEN). A non-limiting example of a suitable PEN is 5 mil thick KALADEX®, a PEN film commercially available from E.I. DuPont de Nemours, Wilmington, Delaware, which 25 is coated with gold by ROWO Coating, Henbolzheim, Germany.

Electrodes 14, 16, 18 are created or isolated from conductor 13 on first surface 24 of substrate 12. Non-limiting examples of a suitable electrical conductor 13 include aluminum, carbon (such as graphite), cobalt, copper, gallium, gold, indium, iridium, iron, lead, magnesium, mercury (as an amalgam), nickel, niobium, osmium, 30 palladium, platinum, rhenium, rhodium, selenium, silicon (such as highly doped polycrystalline silicon), silver, tantalum, tin, titanium, tungsten, uranium, vanadium, zinc, zirconium, mixtures thereof, and alloys, oxides, or metallic compounds of these elements. Preferably, electrical conductor 13 is selected from the following materials: gold, platinum, palladium, iridium, or alloys of these metals, since such noble metals

and their alloys are unreactive in biological systems. Most preferably, electrical conductor 13 is gold.

Electrodes 14, 16, 18 are isolated from the rest of the electrical conductor 13 by laser ablation. See Fig. 3. Techniques for forming electrodes on a surface using 5 laser ablation are known. See, for example, U.S. Patent Application No. 09/411,940, filed October 4, 1999, and entitled "LASER DEFINED FEATURES FOR PATTERNED LAMINATES AND ELECTRODE", the disclosure of which is expressly incorporated herein by reference. Preferably, electrodes 14, 16, 18 are created by removing the electrical conductor 13 from an area extending around the 10 electrodes.

Therefore, electrodes 14, 16, 18 are isolated from the rest of the electrically-conductive material on substrate 12 by a gap having a width of about 25  $\mu\text{m}$  to about 500  $\mu\text{m}$ , preferably the gap has a width of about 100  $\mu\text{m}$  to about 200  $\mu\text{m}$ .

Alternatively, it is appreciated that electrodes 14, 16, 18 may be created by laser 15 ablation alone on substrate 12. It is appreciated that while laser ablation is the preferred method for forming electrodes 14, 16, 18 given its precision and sensitivity, other techniques such as lamination, screen-printing, or photolithography may be used in accordance with this disclosure.

As shown in Fig. 2, electrodes 14, 16, 18 cooperate with one another to define 20 first and second electrode arrays 76, 78 and leads 80 that extend away from the first and second arrays 76, 78. Leads 80 extend from the arrays 76, 78 to contacts 74 at the second end 30 of the electrode-support substrate 12. It is appreciated that the leads extending from the arrays can be formed to have many lengths and extend to a variety of locations on the electrode-support substrate 12. It is appreciated that the 25 configuration of the electrode arrays, the number of electrodes, as well as the spacing between the electrodes may vary in accordance with this disclosure and that a greater than two arrays may be formed as will be appreciated by one of skill in the art.

Spacer substrate 20 of biosensor 10 includes a first member 40 extending between the edges 32, 34 of the electrode-support substrate 12 and second and third 30 members 42, 44 spaced-apart from the first member 40. Each member 40, 42, 44 includes an upper surface 46 and a lower surface 48 facing substrate 12. In addition, the first member 40 includes an inner edge 50 facing the second and third members 42, 44 and an outer edge 52. Second and third members 42, 44 include a first edge 54

facing the first member 40 and a second edge 56. The second edge 56 of the second and third members 42, 44 face one another.

When spacer substrate 20 is coupled to substrate 12, as shown in Figs. 1B and 4, the electrode arrays 76, 78 are positioned to lie between the first member 40 and the second and third members 42, 44. In addition, second and third members 42, 44 lie adjacent to border 38 of notch 36. Spacer substrate 20 is formed of a flexible polymer and preferably from a flexible polymer and preferably from a polymer such as an adhesive coated polyethylene terephthalate (PET)—polyester. A non-limiting example of a suitable PET is 3 mil (75 um) thick white PET film both sides of which are coated with a pressure-sensitive adhesive (Product # ARcare 8877) commercially available from Adhesives Research, Inc. Glen Rock, Pennsylvania.

It is appreciated that spacer substrate 20 may be constructed of a variety of materials and may be coupled to the substrate 12 and the cover substrate 22 using a wide variety of commercially available adhesives. Additionally, when surface 24 of substrate 12 is exposed and not covered by electrical conductor 13, spacer substrate 20 may be coupled to substrate 12 by welding (heat or ultrasonic) in accordance with this disclosure. It is also appreciated that first surface 24 of substrate 12 may be printed with, for example, product labeling or instructions for use in accordance with this disclosure.

Cover substrate 22 is coupled to upper surface 46 of spacer substrate 20. See Fig. 1B. Cover substrate 22 includes an inner surface 58 facing spacer substrate 20 and an outer surface 60. In addition, cover 22 includes opposite first and second ends 62, 64 and edges 66, 68 extending between the first and second ends 62, 64. First end 62 includes a notch 70. A generally concave-shaped border 72 defines notch 70. It is appreciated that the notch can take on a variety of shapes and sizes in accordance with this disclosure. When biosensor 10 is assembled, cover 22 cooperates with the spacer support 20 and the electrode-support 12 to define a capillary channel 82.

Cover substrate 22 is generally rectangular in shape, it is appreciated, however, that the cover may be formed in a variety of shapes and sizes in accordance with this disclosure. Cover substrate 22 is formed from a flexible polymer and preferably from a polymer such as a polyester or polyimide. A non-limiting example of a suitable polymer is 3.9 mil (99 um) thick 3M hydrophilic Polyester film (3M Product #9971) commercially available from 3M Healthcare, St. Paul, MN.

Referring now to Figs. 1A and 1B, the capillary channel 82 is generally linear in shape and includes a sample inlet 84 and spaced-apart ends 86. As shown in Fig. 1A, ends 86 are positioned between edges 32, 66 and 34, 68 respectively. It is appreciated that while biosensor 10 illustratively includes ends 86 that serve as air outlets, it is appreciated that biosensor 10 can be formed to include any number of air outlets in accordance with this disclosure. The capillary channel 82 extends between borders 38, 72 and edges 32, 66 and 34, 68 respectively. Channel is also defined by inner edge 50 of first member 40 of the spacer substrate 20 and the first edges 54 of the second and third members 42, 44. See, Fig. 4. Therefore, when biosensor 10 is assembled, channel 82 extends across the electrode arrays 76, 78. See, for example Fig. 1B.

Electrochemical reagents 88 are positioned on first and second arrays 76, 78. Reagents 88 provide electrochemical probes for specific analytes. The choice of specific reagents 88 depend on the specific analyte or analytes to be measured, and are well known to those of ordinary skill in the art. An example of a reagent that may be used in biosensor 10 of the present invention is a reagent for measuring glucose from a whole blood sample. A non-limiting example of a reagent for measurement of glucose in a human blood sample contains 62.2 mg polyethylene oxide (mean molecular weight of 100-900 kilo Daltons), 3.3 mg NATROSOL 244M, 41.5 mg AVICEL RC-591 F, 89.4 mg monobasic potassium phosphate, 157.9 mg dibasic potassium phosphate, 437.3 mg potassium ferricyanide, 46.0 mg sodium succinate, 148.0 mg trehalose, 2.6 mg TRITON X-100 surfactant, and 2,000 to 9,000 units of enzyme activity per gram of reagent. The enzyme is prepared as an enzyme solution from 12.5 mg coenzyme PQQ and 1.21 million units of the apoenzyme of quinoprotein glucose dehydrogenase. This reagent is further described in U.S. Patent No. 5,997,817, the disclosure of which is expressly incorporated herein by reference.

Non-limiting examples of enzymes and mediators that may be used in measuring particular analytes in biosensor 10 are listed below in Table I.

TABLE I

Analyte	Enzymes	Mediator (Oxidized Form)	Additional Mediator
Glucose	Glucose Dehydrogenase and Diaphorase	Ferricyanide	
Glucose	Glucose-Dehydrogenase (Quinoprotein)	Ferricyanide	
Cholesterol	Cholesterol Esterase and Cholesterol Oxidase	Ferricyanide	2,6-Dimethyl-1,4-Benzoquinone 2,5-Dichloro-1,4-Benzoquinone or Phenazine Ethosulfate
HDL Cholesterol	Cholesterol Esterase and Cholesterol Oxidase	Ferricyanide	2,6-Dimethyl-1,4-Benzoquinone 2,5-Dichloro-1,4-Benzoquinone or Phenazine Ethosulfate
Triglycerides	Lipoprotein Lipase, Glycerol Kinase, and Glycerol-3-Phosphate Oxidase	Ferricyanide or Phenazine Ethosulfate	Phenazine Methosulfate
Lactate	Lactate Oxidase	Ferricyanide	2,6-Dichloro-1,4-Benzoquinone
Lactate	Lactate Dehydrogenase and Diaphorase	Ferricyanide Phenazine Ethosulfate, or Phenazine Methosulfate	
Lactate Dehydrogenase	Diaphorase	Ferricyanide	Phenazine Ethosulfate, or Phenazine Methosulfate
Pyruvate	Pyruvate Oxidase	Ferricyanide	
Alcohol	Alcohol Oxidase	Phenylenediamine	
Bilirubin	Bilirubin Oxidase	1-Methoxy-Phenazine Methosulfate	
Uric Acid	Uricase	Ferricyanide	

In some of the examples shown in Table I, at least one additional enzyme is used as a reaction catalyst. Also, some of the examples shown in Table I may utilize 5 an additional mediator, which facilitates electron transfer to the oxidized form of the mediator. The additional mediator may be provided to the reagent in lesser amount than the oxidized form of the mediator. While the above assays are described, it is contemplated that current, charge, impedance, conductance, potential, or other electrochemically indicated property of the sample might be accurately correlated to

the concentration of the analyte in the sample with biosensor 10 in accordance with this disclosure.

A plurality of biosensors 10 are typically packaged in a vial, usually with a stopper formed to seal the vial. It is appreciated, however, that biosensors 10 may be  
5 packaged individually, or biosensors can be folded upon one another, rolled in a coil, stacked in a cassette magazine, or packed in blister packaging.

Biosensor 10 is used in conjunction with the following:

1. a power source in electrical connection with contacts 74 and capable of supplying an electrical potential difference between electrodes 14, 16 and 18, 16  
10 sufficient to cause diffusion limited electro-oxidation of the reduced form of the mediator at the surface of the working electrode; and
  2. a meter in electrical connection with contacts 74 and capable of measuring the diffusion limited current produced by oxidation of the reduced form of the mediator with the above-stated electrical potential difference is applied.
- 15 The meter will normally be adapted to apply an algorithm to the current measurement, whereby an analyte concentration is provided and visually displayed. Improvements in such power source, meter, and biosensor system are the subject of commonly assigned U.S. Pat. No. 4,963,814, issued Oct. 16, 1990; U.S. Pat. No. 4,999,632, issued Mar. 12, 1991; U.S. Pat. No. 4,999,582, issued Mar. 12, 1991; U.S.  
20 Pat. No. 5,243,516, issued Sep. 7, 1993; U.S. Pat. No. 5,352,351, issued Oct. 4, 1994; U.S. Pat. No. 5,366,609, issued Nov. 22, 1994; White et al., U.S. Pat. No. 5,405,511, issued Apr. 11, 1995; and White et al., U.S. Pat. No. 5,438,271, issued Aug. 1, 1995, the disclosures of each of which are expressly incorporated by reference.

Many fluid samples may be analyzed. For example, human body fluids such  
25 as whole blood, plasma, sera, lymph, bile, urine, semen, cerebrospinal fluid, spinal fluid, lacrimal fluid and stool specimens as well as other biological fluids readily apparent to one skilled in the art may be measured. Fluid preparations of tissues can also be assayed, along with foods, fermentation products and environmental substances, which potentially contain environmental contaminants. Preferably, whole  
30 blood is assayed with this invention.

To manufacture biosensor 10 a roll of metallized electrode support material 90 is fed as shown by arrow 92 in Fig. 5 through guide rolls into an ablation/washing and drying station 94. A laser system capable of ablating support 14 is known to those of ordinary skill in the art. Non-limiting examples of which include excimer lasers, with

the pattern of ablation controlled by mirrors, lenses, and masks. A non-limiting example of such a custom fit system is the LPX-400, LPX-300 or LPX-200 commercially available from LPKF Laser Electronic GmbH, of Garbsen, Germany.

In the laser station 94, the metallic layer of the metallized film is ablated in a  
5 pre-determined pattern, to form a ribbon of isolated electrode sets on the electrode support material. To ablate electrodes 14, 16, 18 in 50 nm thick gold conductor 13, 90 mJ/cm<sup>2</sup> energy is applied. It is appreciated, however, that the amount of energy required may vary from material to material, metal to metal, or thickness to thickness.  
10 The ribbon is then passed through more guide rolls, with a tension loop and through an optional inspection system where both optical and electrical inspection can be made. The system is used for quality control in order to check for defects.

Upon leaving station 94, the metallized film is fed, as shown by arrow 96, into a reagent dispensing station 98. Reagents that have been compounded are fed, as shown by arrow 100, into dispensing station 98 where they are line-coated to cover  
15 part of the channel 82 using a custom-fit commercially available system from Iwashita Engineering Incorporated, Tokyo, Japan.

Alternatively, reagents that have been compounded are discretely applied in a liquid form to the center of respective first and second arrays 76, 78 using a custom-fit commercially available system from Flulogic Systems, Espoo, Finland. When  
20 discrete application of reagents is selected, it is beneficial to create at least one recess (not shown) in the support substrate 12 surrounding the desired reagent-application site to retain the reagents 88 within the circumscribed reagent-application site boundary. Reagent application techniques are well known to one of ordinary skill in the art as described in U.S. Patent No. 5,762,770, the disclosure of which is expressly incorporated herein by reference. It is appreciated that the reagents may be applied to  
25 arrays 76, 78 in a liquid or other form and dried or semi-dried onto arrays 76, 78 in accordance with this disclosure.

In a separate process, a double-sided pressure-sensitive film with dual release liners 102 is fed, as shown by arrow 104, into a lamination & kiss-cutting station 112.  
30 At the same time, a roll of cover substrate material 106 is fed over a guide roll 110 as shown by arrow 108 into the station 112. In the station 112, the release liner is removed from the upper surface 46 as shown by arrow 114, from the station 112 and rewound over guide roll 116 into a roll 118 for discard. In the station 112, the upper surface 46 of the film 102 is applied to the inner surface 58 of cover substrate material

106. Next, the film is kiss cut and a portion of the film is removed leaving behind first member 40 and a second/third member strip (not shown) coupled to the cover material. The cover material is exposed between the first member 40 and the second/third member.

5        The cover material/spacer substrate subassembly is fed, as shown by arrow 122, into a sensor lamination & cut/pack station 124. The reagent-coated electrode-support substrate material is fed from station 98, as shown by arrow 120, into the sensor lamination & cut/pack station 124 as well. The remaining release liner is removed from the spacer substrate and the spacer substrate is positioned on the

10      electrode-support substrate material so that the electrode arrays 76, 78 lie between the first member 40 and the second/third member strip. Once assembled, notches 36, 70 are punched through electrode-support substrate material, the second/third member of the spacer substrate, and the cover substrate material. This cut forms the sample inlet 84 to the capillary channel 82.

15      Next, the resulting assembled material is cut to form individual biosensors 10, which are sorted and packed into vials, each closed with a stopper, to give packaged biosensor strips.

In use, a user of biosensor 10 places a finger having a blood collection incision against borders 38, 72 of notches 36, 70. Capillary forces pull a liquid blood sample flowing from the incision into the sample inlet 84 and through the capillary channel 82 across the reagents 88 and the arrays 76, 78. The liquid blood sample dissolves the reagents and engages the arrays 76, 78 where the electrochemical reaction takes place.

20      In use, after the reaction is complete, a power source (e.g., a battery) applies a potential difference between the electrodes 14, 16 and 18, 16 respectively. When the potential difference is applied, the amount of oxidized form of the mediator at the reference electrode and the potential difference must be sufficient to cause diffusion-limited electro-oxidation of the reduced form of the mediator at the surface of the working electrode. A current measuring meter (not shown) measures the diffusion-limited current generated by the oxidation of the reduced form of the mediator at the

25      30      surface of the working electrode.

The measured current may be accurately correlated to the concentration of the analyte in sample when the following requirements are satisfied:

1. The rate of oxidation of the reduced form of the mediator is governed by the rate of diffusion of the reduced form of the mediator to the surface of the working electrode.

2. The current produced is limited by the oxidation of reduced form of the mediator at the surface of the working electrode.

The processes and products described above include disposable biosensor 10 especially for use in diagnostic devices. Also included, however, are electrochemical sensors for non-diagnostic uses, such as measuring an analyte in any biological, environmental, or other sample. As discussed above, biosensor 10 can be

10 manufactured in a variety of shapes and sizes and be used to perform a variety of assays, non-limiting examples of which include current, charge, impedance conductance, potential or other electrochemical indicative property of the sample applied to biosensor.

Although the invention has been described in detail with reference to a  
15 preferred embodiment, variations and modifications exist within the scope and spirit of the invention, on as described and defined in the following claims